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Starch - protein extraction and separation it from green pea

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Abstract

Legume is important food in healthy nutrition as a source of complex carbohydrates, proteins and dietary fibre, having significant amounts of vitamins and minerals, and high energetic value. It contains high amount of slowly digestible starches (SDS), and legumes have relatively low glycemic index (GI ~ 30).

There are many varieties of pea with different properties and composition, smooth or wrinkled seeded. Pea starch contains more amylose than commonly used starches therefore it is suitable material for producing biodegradable packages, paper and corrugated boards.

Garden (green) pea, cultivar Kashan (Kashan green pea), was tested for starch and protein extraction in laboratory. Hulled pea contained 31.2 % of starch with high content of amylose, 32.3 % of proteins and 2.0 % of lipids in dry matter.

The results were obtained with 8 experiments coming from dehulled and unhulled pea. At first the seeds were either milled (and the flour was sieved in some experiments) and then mixed with water or a liquidizer was used to disintegrate the seeds in water. After that, proteins were dissolved at pH 9.0 adjusted using 1M NaOH solution. Starch and fibre were separated using a laboratory centrifuge. The procedure was repeated 2x. The dissolved proteins were acidified at the isoeletric point pH 4.5 and the precipitate was re-dispersed using 1M NaOH solution and then again precipitated by an acid.

As expected, the isolated starch coming from dehulled seeds was of a higher purity compared with the unhulled ones. The best purity - 78% of starch in dry matter with the lowest content of proteins (3.7 %) and lipids (0.35 %) in dry matter was detected when using soft fraction of flour from dehulled seeds. Higher yield of proteins was obtained from the fraction of flour retained on a sieve.

Keywords: extraction, pea, protein, starch.

Introduction

Legumes are dicotyledonous seeds of plants that belong to the family Leguminosae. The relative advantages of this crop are: it can be easily harvested using machinery; it is a valuable break-crop in a rotation which is dominated by cereals such as oats, barley, etc.; and under poor soil conditions, legume crops play an important role in soil fertility improvement due to their ability to fix atmospheric nitrogen. (Davis 1993, Zadorin and Isaev 1999)

In general, legume is a source of complex carbohydrates, proteins and dietary fiber, having significant amounts of vitamins and minerals. (Costa et al. 2006). There are however also antinutrient matters in legume (flatulent oligosaccharides such as verbascose and stachyose, lectins, protease inhibitors and others) which can be minimized by washing, heat processing and/or by germination. (Nikolopoulou 2007, Kadlec et al. 2006)

Field pea is utilized as follows: (1) whole or split in soups and stews; (2) hulls in high fibre breads; (3) pea protein in human food and in hog rations as an alternative protein source to soy and canola meal, and (4) pea starch in production of adhesives and carbonless paper. (Ratnayake et al., 2001). Pea starch is mainly available as a by product of protein extraction. Therefore, it is considered to be a relatively cheap source of starch compared to corn, wheat and potato starches.

In the species P. sativum L. , two different seed phenotypes exist, namely, smooth (with a smooth seed surface) and wrinkled pea (wrinkled seed surface). The two types are genetically different and produce characteristic starches with different granular morphologies and characteristics.

The granule size of normal pea (smooth pea) starch is variable and ranges from 2–40 µm. Most of the granules are oval, although spherical, round, elliptical and irregularly shaped granules are also found. They are observed two distinct populations in the size distribution of smooth pea starch granules (large granules with diameters ranging from 15–30 µm and small granules with diameters ranging from 2–8 µm). (Ratnayake et al., 2002)

Wrinkled pea starch appears to be a mixture of simple and compound granules, the latter being composed of 3–10 sub-units fused together (Collona et al. 1982). According to Bertroft (1993) large granules of wrinkled pea are cracked and composed of 4–6 loosely associated pieces in a ring formation with a diameter ranging from 17–30 µm.

Generally, legume starches are characterized by a high amylose content. Normal pea (smooth pea) and wrinkled pea starches differ mainly in their amylose/amylopectin ratios and by the presence of an intermediate material of low molecular weight in the latter. The amylose content of smooth pea, pea mutants and wrinkled pea starches ranges from 33.1–49.6%, 8– 72%, and 60.5–88%, respectively. (Ratnayake et al., 2002) Therefore pea starch is suitable material for producing biodegradable packages, paper and corrugated boards.

Pea starch has been used widely in the processed meat industry where heat and mechanical stability are of great interest. In canned foods, cooked sausages, pates, and other foods, it can substitute for traditional starches with excellent results.

The starch has unique gelatinization properties (no breakdown at elevated temperatures); consequently, the starch can be used at a reduced concentration to lower the caloric content in soups, gravies, and the like. As a thickening agent in soups and sauces and in many other products, pea starch gives a pleasant mouth feel. Heat stability, excellent expansion properties and marked resistance to mechanical shear are important in extruded products. (Czuchajowska and Pomeranz, 1994).

Pea protein might be used as a food supplement because of a very good composition of essential and nonessential amino acids. It can be a useful protein source for vegetarians; except this it doesn’t contain cholesterol and lactose compared with milk protein.

But the separation of starches from peas is difficult, owing to the presence of insoluble flocculent proteins and fine fiber, which decreases sedimentation and co-settles with the starch to give a brownish deposit. (Ratnayake et al., 2002)

The objective of our research was to compare different methods of pea starch and protein isolation as to purities and yields of isolated fractions.

Materials and methods

Seeds of green garden pea cultivar kashan were kindly provided. This crop is a medium-ripening type; it is suitable for industrial processing as well as for small farmers.

The starch and protein fractions were separated from pea flour and pea seeds using modified procedures, as proposed by Czuchajowska a Pomeranz (1994) and Chavan et al. (2001), respectively.

A pea suspension used for separation was prepared from 100 g of pea flour or seeds and 220 ml distilled water. The initial solid material was prepared as follows:

(Exp. 1) Flour prepared grinding of unhulled seeds; (Exp. 2) The part of flour passed through 0.08 mm sieve; (Exp. 3) Oversize on 0.08 mm sieve;

(Exp. 4) Flour prepared from dehulled seeds (pea seeds were submerged in distilled water overnight, and then dehulled, dried and ground)

(Exp. 5) The part of flour prepared from dehulled seeds, passed through 0.08 mm sieve; (Exp. 6) Oversize on 0.08 mm sieve;

(Exp. 7) Unhulled seeds;

(Exp. 8) Dehulled seeds (the same way as in (4)).

The suspension when processing the whole seeds was prepared using a kitchen liquidizer (1 min mixing).

The suspension was placed in a beaker and stirred with a paddle agitator for 10 min. The pH was then adjusted to 9.0 using 1 M NaOH and the suspension was kept stirring for 30 min. Then it was centrifuged for 20 min at 9000 rpm. The liquid after separation contained dissolved protein and it was used for separation of the first fraction of proteins.

The separated solid phase containing major part of starch was dispersed in 220 ml of distilled water. The pH of the suspension was again adjusted to 9.0 using 1 M NaOH and kept stirring for 30 min. The suspension was centrifuged in the same way and the liquid was used as the second protein fraction. After that the procedure was repeated to have the third protein fraction.

Separated starch was dried at 40 – 43 °C and then ground into a fine powder.

All of three liquid fractions were processed the same way. They were precipitated by adjusting pH to 4.5 using 1 M HCl. The precipitate was centrifuged for 20 min at 4000 rpm. The supernatant was removed and the precipitate was re-dispersed in distilled water, then pH was adjusted to 9.0 using 1 M NaOH and then again precipitated by adjusting pH to 4.5 and centrifuged. Isolated proteins were dried at 40 – 43 °C. Dry samples were ground into a fine powder.

Total starch was determined using a Total Starch Assay Kit, Megazyme Ireland, by AACC 76-13 method.

Amylose content was determined colourimetrically according to McGrance (1998). The result was compared with the Megazyme amylose/amylopectin assay procedure, utilizing the commercial kit (Megazyme Ireland International, Ltd., Bray, Ireland) followed according to the manufacturer's recommendation.

Protein (Kjeldahl), Moisture and ash were determined according to European standards.

Lipid content was determined by petroleum ether extraction in a Soxterm apparatus.

Results and discussion

The chemical composition of dehulled pea seeds was found as follows: total starch content of 31.2 % (amylose content 83 %), protein content of 32.3 %, and lipid content 2.0 % lipids in dry matter. These values were lower for the unhulled pea seeds containing 30.4 % of starch, 27.8 % of proteins a 1.8 % of lipids in dry matter. These values are near the data of Singer (1997), Wang and Daun (2004) and Davydova et al. (1995).

It was confirmed the expected positive effect of seed hulling on the quality of both products. Isolated starch from experiments with dehulled seeds had always higher purity than the product from unhulled ones. Figure 1 illustrates the starch and protein contents in separated starch fractions.

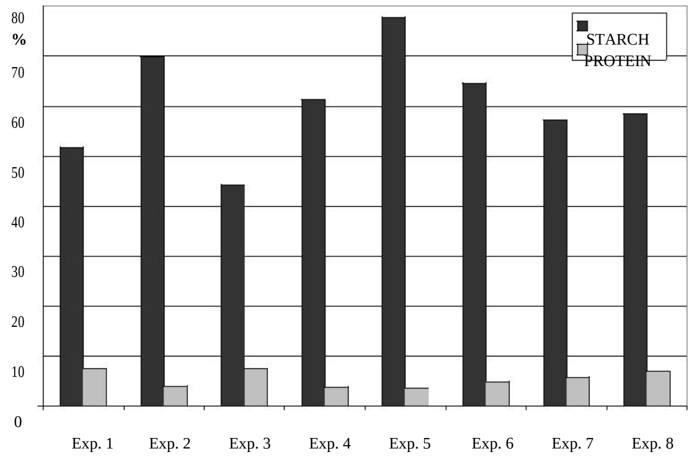

Figure 1: Starch and protein contents in separated starch fractions.

The sieving of the pea flour influenced positively on the separation effect of the protein and starch fractions. The starch products prepared from particles, which passed through 0.08 mm sieve, were of higher purity than fractions prepared from oversized ones - see Fig. 1. The starch separation of highest purity was obtained for dehulled pea flour from particles, which passed through 0.08 mm sieve (Exp. 5). It contained 77.8 % of starch and of 3.7 % proteins. On the other hand the highest yield of isolated protein was obtained from the suspension coming from dehulled pea flour with particles bigger than 80 µm (see Table 1: Exp. 6 – the sum of three parts of protein was found 21.6 g per 100 g flour). These positive findings agree with the statement published in US patent. (Czuchajowska and Pomeranz 1994).

Table 1: Yield and chemical composition of pea protein separate

Sample	1 part			2 part			3 part		
	Weight (g)	Protein (%)	Starch (%)	Weight (g)	Protein (%)	Starch (%)	Weight (g)	Protein (%)	Starch (%)
Exp. 1	12.91	70.96	0.38	4.52	80.89	0.11	0.89	76.17	0.96
Exp. 2	9.73	75.34	0.27	6.85	80.17	0.27	1.56	75.58	0.43
Exp. 3	10.73	79.13	0.49	6.99	72.84	1.98	1.98	80.96	0.75
Exp. 4	16.24	70.88	0.78	5.57	75.01	0.34	1.21	69.48	1.79
Exp. 5	10.98	78.40	0.29	4.59	77.41	0.24	1.12	73.63	1.15
Exp. 6	13.91	75.02	0.62	6.40	80.29	0.20	1.32	70.15	2.37
Exp. 7	5.58	79.83	0.10	2.32	78.73	0.82	2.34	75.74	3.83
Exp. 8	4.62	78.03	0.59	2.57	81.81	0.48	3.40	82.31	0.47

When comparing wet and dry methods of pea disintegration, the higher yields (31 – 57 g per 100 g flour) and purities were obtained for dry milling. The yield after wet mixing was low - only about 27 g per 100 g (see Table 2).

Table 2: Yield and chemical composition of pea starch separate

Sample	Weight (g)	Dry substance DS (%)	Starch (% DS)	Protein (% DS)	Lipid (% DS)	Ash (%¨DS)
Exp. 1	46.08	95.82	51.78	7.39	0.20	1.82
Exp. 2	42.58	95.51	69.87	3.91	0.51	1.55
Exp. 3	42.89	96.49	44.30	7.46	-	1.76
Exp. 4	35.97	95.82	61.27	3.82	0.27	1.94
Exp. 5	31.76	95.75	77.84	3.66	0.35	1.46
Exp. 6	31.86	95.44	64.63	4.96	-	1.79
Exp. 7	27.61	96.66	57.16	5.76	-	2.16
Exp. 8	26.63	96.57	58.45	6.99	-	2.39

Conclusion

The aim of the work was to compare different methods of pea starch and protein separation as to purities and yields of separated fractions from seeds of garden pea, cultivar kashan (of amylose content in starch 83 %). The fractions were isolated from pea flour and pea seeds using modified procedures, as proposed by Czuchajowska a Pomeranz (1994) and Chavan et al. (2001), respectively.

The pH of suspensions prepared in a different way was adjusted to 9.0 using 1 M NaOH to dissolve proteins which were then precipitated at pH 4.5.

The starch separation of highest purity was obtained for dehulled pea flour from particles, which passed through 0.08 mm sieve. It contained 77.8 % of starch and of 3.7 % proteins. Highest yield of separated protein was obtained from the suspension coming from dehulled pea flour with particles bigger than 80 µm (21.6 g per 100 g flour). Industrial separation comprising refining centrifuges and hydrocyclones could improve the obtained data.

References

Bertoft, E., Manelius, R., Qin, Z. (1993). Studies on the structure of pea starches. Part 2. α-Amylolysis of granular wrinkled pea starch. Starch/Stärke 45: 258–263.

Chavan, U.D., McKenzie, D.B., Shahidi, F. (2001). Functional properties of protein isolates from beach pea (Lathyrus maritimus L.). Food Chemistry 74: 177-187.

Colonna, P., Buléon, A., LeMaguer, M., Mercier, C. (1982). Pisum sativum and Vicia faba carbohydrates: Part IV – Granular structure of wrinkled pea starch. Carbohydr. Polym. 2: 43– 59.

Costa, G.E.A., Monici, K.S.Q., Reis, S.M.P.M., & Oliveira, A.C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chemistry, 94: 327-330.

Czuchajowska, Z., Pomeranz, Y. (1994). Fractionation of legumes to obtain pure starch and a protein concentrate. U.S.Pat. č. 5364471.

Davies, D. R. (1993). The pea crop, in: Peas, genetics, molecular biology and biotechnology. (Eds. R. Casey, D. R. Davies). CAB International, Oxon, pp. 1–12.

Davydova, N.I., Leont′ev, S.P., Genin, Ya.V., Sasov, A.Yu., Bogracheva, T.Ya. (1995). Some physico-chemical properties of smooth pea starches. Carbohydrate Polymers, 27: 109-115.

Kadlec, P., Dostalova, J., Houska, M., Strohalm, J., Bubnik, Z. (2006). Evaluation of α-galactosides decrease during storage of germinated pea seeds treated by high pressure. Journal of Food Engineering 64: 445-448.

Nikolopoulou, D., Grigorakis, K., Stasini, M., Alexis, M.N., Iliadis, K. (2007). Differences in chemical composition of field pea (Pisum sativum) cultivars: Effects of cultivation area and year. Food Chemistry, 103: 847–852.

McGrance, S.J., Cornell, H.J., Rix, C.J. (1998). A simple and rapid colorimetric method for the determination of amylose in starch products. Starch/Stärke, 50: 158-163.

Ratnayake, W.S., Hoover, R., Shahidi, F., Perera, C., Jane, J. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (Pisum sativum L.) cultivars. Food Chemistry, 74: 189-202.

Ratnayake, W. S., Hoover, R., Warkentin, T. (2002). Pea Starch: Composition, Structure and Properties – A Review. Starch.54: 217-234.

Singer, M. (1997). Hrachovy shkrob – potencionalny zdroj amylosy. (3), 14-15.

Wang, N., Daun, J.K. (2004). Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (Pisum sativum). Journal of the Science of Food and Agriculture, 84: 1021-1029.

Zadorin, A., Isaev, A. (1999). Pea cultivation in Russia. Grain Legumes 25: 26–27.